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NEWS	11	SEP 01	INPADOC: New family current-awareness alert (SDI) available
NEWS	12	SEP 01	New pricing for the Save Answers for SciFinder Wizard within STN Express with Discover!
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=> s Marinomonas or Marinomonas species or Marinomonas protea
L1          171 MARINOMONAS OR MARINOMONAS SPECIES OR MARINOMONAS PROTEA

=> ((Anti-freeze or antifreeze or anti freeze) (w) protein))
UNMATCHED RIGHT PARENTHESIS 'PROTEIN))'
The number of right parentheses in a query must be equal to the
number of left parentheses.

=> (Anti-freeze or antifreeze or anti freeze) (w) protein
L2         2756 (ANTI-FREEZE OR ANTIFREEZE OR ANTI FREEZE) (W) PROTEIN

=> L1 and L2
L3            1 L1 AND L2

=> Pseudomonas or Pseudomonas (w) species
L4       307104 PSEUDOMONAS OR PSEUDOMONAS (W) SPECIES

=> L2 and L4
L5           49 L2 AND L4

=> food (w) product or food
L6      1403514 FOOD (W) PRODUCT OR FOOD

=> (food (w) product) or food
L7      1403514 (FOOD (W) PRODUCT) OR FOOD

=> L2 and L7
L8           149 L2 AND L7
```

```
=> L1 and L8
L9            1 L1 AND L8

=> L4 and L8
L10           2 L4 AND L8

=> dup rem L5
PROCESSING COMPLETED FOR L5
L11           23 DUP REM L5 (26 DUPLICATES REMOVED)

=> L1 same L8
MISSING OPERATOR L1 SAME
The search profile that was entered contains terms or
nested terms that are not separated by a logical operator.

=> L1 with L8
MISSING OPERATOR L1 WITH
The search profile that was entered contains terms or
nested terms that are not separated by a logical operator.

=> L1 (p) L8
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L1 (P) L43'
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L2 (P) L44'
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L3 (P) L45'
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L4 (P) L46'
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L5 (P) L47'
L12           1 L1 (P) L8

=> L4 (p) L8
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L19 (P) L43'
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L20 (P) L44'
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L21 (P) L45'
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L22 (P) L46'
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L23 (P) L47'
L13           2 L4 (P) L8

=> dis L1

L1   ANSWER 1 OF 171  BIOSIS  COPYRIGHT (c) 2004 The Thomson Corporation.  on
     STN
AN   2004:347362  BIOSIS
DN   PREV200400349251
TI   Detailed structure of lipid A isolated from lipopolysaccharide from the
     marine proteobacterium Marinomonas vaga ATCC 27119T.
AU   Krasikova, Inna N. [Reprint Author]; Kapustina, Natalie V.; Isakov,
     Vladimir V.; Dmitrenok, Andrey S.; Dmitrenok, Pavel S.; Gorshkova, Natalie
     M.; Solov'eva, Tamara F.
CS   Pacific Inst Bioorgan ChemFar Eastern Branch, Russian Acad Sci, 159,
     Vladivostok, 690022 22, Russia
     innakras@piboc.dvo.ru
SO   European Journal of Biochemistry, (July 2004) Vol. 271, No. 14, pp.
     2895-2904. print.
     ISSN: 0014-2956 (ISSN print).
DT   Article
```

LA English
ED Entered STN: 18 Aug 2004
Last Updated on STN: 18 Aug 2004

=> dis L1 kwic

L1 ANSWER 1 OF 171 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN
TI Detailed structure of lipid A isolated from lipopolysaccharide from the marine proteobacterium **Marinomonas** vaga ATCC 27119T.
AB The chemical structure of a novel lipid A, the major component of the lipopolysaccharide from the marine gamma-proteobacterium **Marinomonas** vaga ATCC 27119T, was determined by compositional analysis, NMR spectroscopy, and MS. It was found to be beta-1,6-glucosaminobiose 1-phosphate acylated. . .
ORGN Classifier
Pseudomonadaceae 06508
Super Taxa
Gram-Negative Aerobic Rods and Cocci; Eubacteria; Bacteria; Microorganisms
Organism Name
Marinomonas vaga (species): pathogen, strain-ATCC 27119
Taxa Notes
Bacteria, Eubacteria, Microorganisms

=> dis L1 abs kwic

L1 ANSWER 1 OF 171 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN
AB The chemical structure of a novel lipid A, the major component of the lipopolysaccharide from the marine gamma-proteobacterium **Marinomonas** vaga ATCC 27119T, was determined by compositional analysis, NMR spectroscopy, and MS. It was found to be beta-1,6-glucosaminobiose 1-phosphate acylated with (R)-3-(dodecanoyl(dodecenoyl)oxy)decanoic acid {C10 : 0 (3O-C12 : 0 (3O-C12 : 1))} or (R)-3-(decanoyloxy)decanoic acid (C10 : 0 (3O-C10 : 0)), (R)-3-hydroxydecanoic acid (C10 : 0 (3OH)), and (R)-3-((R)-3-hydroxydecanoyloxy)decanoic acid (C10 : 0 {3O-(C10 : 0 (3OH))}) at the 2, 3, and 2' positions, respectively. It showed low lethal toxicity, which is probably related to specific structural attributes. The absence of a fatty acid at the 3' position and a phosphoryl group at the 4' position and also the presence of an amide-linked (R)-3-hydroxyalkanoic acid that is further O-acylated with another (R)-3-hydroxyalkanoic acid, distinguish M. vaga lipid A from other such molecules.
TI Detailed structure of lipid A isolated from lipopolysaccharide from the marine proteobacterium **Marinomonas** vaga ATCC 27119T.
AB The chemical structure of a novel lipid A, the major component of the lipopolysaccharide from the marine gamma-proteobacterium **Marinomonas** vaga ATCC 27119T, was determined by compositional analysis, NMR spectroscopy, and MS. It was found to be beta-1,6-glucosaminobiose 1-phosphate acylated. . .
ORGN Classifier
Pseudomonadaceae 06508
Super Taxa
Gram-Negative Aerobic Rods and Cocci; Eubacteria; Bacteria; Microorganisms
Organism Name
Marinomonas vaga (species): pathogen, strain-ATCC 27119
Taxa Notes
Bacteria, Eubacteria, Microorganisms

=> dis L9 abs kwic

L9 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2004 ACS on STN
AB The invention relates to a process for preparing a novel anti-freeze peptide and to the peptides obtained from bacteria from an aqueous low-temperature environment, such as **Marinomonas protea** and a novel Pseudomonas species. These anti-freeze peptides can suitably be incorporated in frozen **food products** such as frozen vegetables and frozen confectionery such as ice cream.
TI Processes and organisms for the production of **anti-freeze proteins**
AB . . . for preparing a novel anti-freeze peptide and to the peptides obtained from bacteria from an aqueous low-temperature environment, such as **Marinomonas protea** and a novel Pseudomonas species. These anti-freeze peptides can suitably be incorporated in frozen **food products** such as frozen vegetables and frozen confectionery such as ice cream.
ST **antifreeze protein** fermn **Marinomonas** Pseudomonas; **food antifreeze protein**; sequence **antifreeze protein**
IT Ice cream
(**anti-freeze proteins** for use in)
IT Proteins, specific or class
RL: BMF (Bioindustrial manufacture); BPN (Biosynthetic preparation); FFD (Food or feed use); PRP (Properties); PUR (Purification or recovery); BIOL (Biological study); PREP (Preparation); USES (Uses)
(antifreeze; processes and organisms for the production of **anti-freeze proteins**)
IT Vegetable
(frozen; **anti-freeze proteins** for use in)
IT Fermentation
Marinomonas protea
Pseudomonas synxantha
(processes and organisms for the production of **anti-freeze proteins**)
IT Frozen **foods**
(vegetables; **anti-freeze proteins** for use in)
IT 346420-90-4P
RL: BMF (Bioindustrial manufacture); BPN (Biosynthetic preparation); FFD (Food or feed use); PRP (Properties); PUR (Purification or recovery); BIOL (Biological study); PREP (Preparation); USES (Uses)
(amino acid sequence; processes and organisms for the production of **anti-freeze proteins**)
IT 344824-54-0 346011-36-7
RL: PRP (Properties)
(nucleotide sequence; processes and organisms for the production of **anti-freeze proteins**)
IT 344824-56-2 346013-83-0, 9: PN: WO0144275 FIGURE: 3 unclaimed DNA
RL: PRP (Properties)
(unclaimed nucleotide sequence; processes and organisms for the production of **anti-freeze proteins**)
IT 225675-43-4 344824-55-1
RL: PRP (Properties)
(unclaimed sequence; processes and organisms for the production of **anti-freeze proteins**)

=> dis L10 1-2, abs kwic

L10 ANSWER 1 OF 2 CAPLUS COPYRIGHT 2004 ACS on STN
AB The invention relates to a process for preparing a novel anti-freeze peptide and to the peptides obtained from bacteria from an aqueous low-temperature environment, such as Marinomonas protea and a novel **Pseudomonas species.** These anti-freeze peptides can suitably be incorporated in frozen **food products** such as frozen vegetables and

frozen confectionery such as ice cream.

TI Processes and organisms for the production of **anti-freeze proteins**

AB . . . peptide and to the peptides obtained from bacteria from an aqueous low-temperature environment, such as Marinomonas protea and a novel **Pseudomonas species.** These anti-freeze peptides can suitably be incorporated in frozen **food products** such as frozen vegetables and frozen confectionery such as ice cream.

ST **antifreeze protein** fermn Marinomonas **Pseudomonas; food antifreeze protein** ; sequence **antifreeze protein**

IT Ice cream
(**anti-freeze proteins** for use in)

IT Proteins, specific or class
RL: BMF (Bioindustrial manufacture); BPN (Biosynthetic preparation); FFD (Food or feed use); PRP (Properties); PUR (Purification or recovery); BIOL (Biological study); PREP (Preparation); USES (Uses)
(antifreeze; processes and organisms for the production of **anti-freeze proteins**)

IT Vegetable
(frozen; **anti-freeze proteins** for use in)

IT Fermentation
Marinomonas protea
Pseudomonas synxantha
(processes and organisms for the production of **anti-freeze proteins**)

IT Frozen **foods**
(vegetables; **anti-freeze proteins** for use in)

IT 346420-90-4P
RL: BMF (Bioindustrial manufacture); BPN (Biosynthetic preparation); FFD (Food or feed use); PRP (Properties); PUR (Purification or recovery); BIOL (Biological study); PREP (Preparation); USES (Uses)
(amino acid sequence; processes and organisms for the production of **anti-freeze proteins**)

IT 344824-54-0 346011-36-7
RL: PRP (Properties)
(nucleotide sequence; processes and organisms for the production of **anti-freeze proteins**)

IT 344824-56-2 346013-83-0, 9: PN: WO0144275 FIGURE: 3 unclaimed DNA
RL: PRP (Properties)
(unclaimed nucleotide sequence; processes and organisms for the production of **anti-freeze proteins**)

IT 225675-43-4 344824-55-1
RL: PRP (Properties)
(unclaimed sequence; processes and organisms for the production of **anti-freeze proteins**)

L10 ANSWER 2 OF 2 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN

AB This paper reviews new developments in methods of freezing (high-pressure freezing, dehydrofreezing and applications of **antifreeze protein** and ice nucleation protein) and thawing (high-pressure and microwave thawing, ohmic thawing and acoustic thawing) for **foods.** With a good understanding of the solid-liquid phase diagram of water, the effects of pressure on **food** freezing-thawing cycles are highlighted. High-pressure freezing promotes uniform and rapid ice nucleation and growth through the whole sample. Dehydrofreezing has been successfully used in freezing of vegetables and fruits with the advantage of less damage to plant texture because of partial water removal before freezing. Recently, studies have been carried out for the biotechnological use of antifreeze and ice nucleation proteins because of their uniqueness in directly improving freezing processes. Thawing under pressure can be achieved at lower temperature than that at atmospheric pressures. Finally microwave, ohmic

and acoustic thawing are described. It is hoped that this paper will attract more research in novel freezing and thawing processes and methods. (C) 2002 Elsevier Science Ltd. All rights reserved.

TI Novel methods for rapid freezing and thawing of **foods** - a review

AB This paper reviews new developments in methods of freezing (high-pressure freezing, dehydrofreezing and applications of **antifreeze protein** and ice nucleation protein) and thawing (high-pressure and microwave thawing, ohmic thawing and acoustic thawing) for **foods**. With a good understanding of the solid-liquid phase diagram of water, the effects of pressure on **food** freezing-thawing cycles are highlighted. High-pressure freezing promotes uniform and rapid ice nucleation and growth through the whole sample. Dehydrofreezing has. . .

ST Author Keywords: acoustic; **antifreeze protein**; dehydrofreezing; freezing; freezing time; **foods**; high pressure; ice crystal; ice nucleation protein; microwave; ohmic; thawing

STP KeyWords Plus (R): HIGH HYDROSTATIC-PRESSURE; BACTERIAL ICE NUCLEATION; **ANTIFREEZE PROTEINS**; ERWINIA-HERBICOLA; TEXTURAL QUALITY; OSMOTIC CONCENTRATION; **PSEUDOMONAS**-SYRINGAE; FROST INJURY; FROZEN **FOODS**; KINU-TOFU

=> dis L12 abs kwic

L12 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2004 ACS on STN

AB The invention relates to a process for preparing a novel anti-freeze peptide and to the peptides obtained from bacteria from an aqueous low-temperature environment, such as **Marinomonas protea** and a novel Pseudomonas species. These anti-freeze peptides can suitably be incorporated in frozen **food products** such as frozen vegetables and frozen confectionery such as ice cream.

TI Processes and organisms for the production of **anti-freeze proteins**

AB . . . for preparing a novel anti-freeze peptide and to the peptides obtained from bacteria from an aqueous low-temperature environment, such as **Marinomonas protea** and a novel Pseudomonas species. These anti-freeze peptides can suitably be incorporated in frozen **food products** such as frozen vegetables and frozen confectionery such as ice cream.

ST **antifreeze protein** fermn **Marinomonas** Pseudomonas; **food antifreeze protein**; sequence **antifreeze protein**

IT Ice cream
(**anti-freeze proteins** for use in)

IT Proteins, specific or class
RL: BMF (Bioindustrial manufacture); BPN (Biosynthetic preparation); FFD (Food or feed use); PRP (Properties); PUR (Purification or recovery); BIOL (Biological study); PREP (Preparation); USES (Uses)
(antifreeze; processes and organisms for the production of **anti-freeze proteins**)

IT Vegetable
(frozen; **anti-freeze proteins** for use in)

IT Fermentation
Marinomonas protea
Pseudomonas synxantha
(processes and organisms for the production of **anti-freeze proteins**)

IT Frozen **foods**
(vegetables; **anti-freeze proteins** for use in)

IT 346420-90-4P
RL: BMF (Bioindustrial manufacture); BPN (Biosynthetic preparation); FFD (Food or feed use); PRP (Properties); PUR (Purification or recovery); BIOL (Biological study); PREP (Preparation); USES (Uses)
(amino acid sequence; processes and organisms for the production of

anti-freeze proteins)
IT 344824-54-0 346011-36-7
RL: PRP (Properties)
(nucleotide sequence; processes and organisms for the production of **anti-freeze proteins**)
IT 344824-56-2 346013-83-0, 9: PN: WO0144275 FIGURE: 3 unclaimed DNA
RL: PRP (Properties)
(unclaimed nucleotide sequence; processes and organisms for the production of **anti-freeze proteins**)
IT 225675-43-4 344824-55-1
RL: PRP (Properties)
(unclaimed sequence; processes and organisms for the production of **anti-freeze proteins**)

=> dis L13 1-2 abs kwic

L13 ANSWER 1 OF 2 CAPLUS COPYRIGHT 2004 ACS on STN
AB The invention relates to a process for preparing a novel anti-freeze peptide and to the peptides obtained from bacteria from an aqueous low-temperature environment, such as Marinomonas protea and a novel **Pseudomonas species.** These anti-freeze peptides can suitably be incorporated in frozen **food products** such as frozen vegetables and frozen confectionery such as ice cream.
TI Processes and organisms for the production of **anti-freeze proteins**
AB . . . peptide and to the peptides obtained from bacteria from an aqueous low-temperature environment, such as Marinomonas protea and a novel **Pseudomonas species.** These anti-freeze peptides can suitably be incorporated in frozen **food products** such as frozen vegetables and frozen confectionery such as ice cream.
ST **antifreeze protein** fermn Marinomonas **Pseudomonas**; **food antifreeze protein** ; sequence **antifreeze protein**
IT Ice cream
(**anti-freeze proteins** for use in)
IT Proteins, specific or class
RL: BMF (Bioindustrial manufacture); BPN (Biosynthetic preparation); FFD (Food or feed use); PRP (Properties); PUR (Purification or recovery); BIOL (Biological study); PREP (Preparation); USES (Uses)
(antifreeze; processes and organisms for the production of **anti-freeze proteins**)
IT Vegetable
(frozen; **anti-freeze proteins** for use in)
IT Fermentation
Marinomonas protea
Pseudomonas synxantha
(processes and organisms for the production of **anti-freeze proteins**)
IT Frozen **foods**
(vegetables; **anti-freeze proteins** for use in)
IT 346420-90-4P
RL: BMF (Bioindustrial manufacture); BPN (Biosynthetic preparation); FFD (Food or feed use); PRP (Properties); PUR (Purification or recovery); BIOL (Biological study); PREP (Preparation); USES (Uses)
(amino acid sequence; processes and organisms for the production of **anti-freeze proteins**)
IT 344824-54-0 346011-36-7
RL: PRP (Properties)
(nucleotide sequence; processes and organisms for the production of **anti-freeze proteins**)
IT 344824-56-2 346013-83-0, 9: PN: WO0144275 FIGURE: 3 unclaimed DNA
RL: PRP (Properties)
(unclaimed nucleotide sequence; processes and organisms for the production

of **anti-freeze proteins**)
IT 225675-43-4 344824-55-1
RL: PRP (Properties)
(unclaimed sequence; processes and organisms for the production of **anti-freeze proteins**)

L13 ANSWER 2 OF 2 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN
AB This paper reviews new developments in methods of freezing (high-pressure freezing, dehydrofreezing and applications of **antifreeze protein** and ice nucleation protein) and thawing (high-pressure and microwave thawing, ohmic thawing and acoustic thawing) for **foods.** With a good understanding of the solid-liquid phase diagram of water, the effects of pressure on **food** freezing-thawing cycles are highlighted. High-pressure freezing promotes uniform and rapid ice nucleation and growth through the whole sample. Dehydrofreezing has been successfully used in freezing of vegetables and fruits with the advantage of less damage to plant texture because of partial water removal before freezing. Recently, studies have been carried out for the biotechnological use of antifreeze and ice nucleation proteins because of their uniqueness in directly improving freezing processes. Thawing under pressure can be achieved at lower temperature than that at atmospheric pressures. Finally microwave, ohmic and acoustic thawing are described. It is hoped that this paper will attract more research in novel freezing and thawing processes and methods. (C) 2002 Elsevier Science Ltd. All rights reserved.
TI Novel methods for rapid freezing and thawing of **foods** - a review
AB This paper reviews new developments in methods of freezing (high-pressure freezing, dehydrofreezing and applications of **antifreeze protein** and ice nucleation protein) and thawing (high-pressure and microwave thawing, ohmic thawing and acoustic thawing) for **foods.** With a good understanding of the solid-liquid phase diagram of water, the effects of pressure on **food** freezing-thawing cycles are highlighted. High-pressure freezing promotes uniform and rapid ice nucleation and growth through the whole sample. Dehydrofreezing has. . .
ST Author Keywords: acoustic; **antifreeze protein**; dehydrofreezing; freezing; freezing time; **foods**; high pressure; ice crystal; ice nucleation protein; microwave; ohmic; thawing
STP KeyWords Plus (R): HIGH HYDROSTATIC-PRESSURE; BACTERIAL ICE NUCLEATION; **ANTIFREEZE PROTEINS**; ERWINIA-HERBICOLA; TEXTURAL QUALITY; OSMOTIC CONCENTRATION; **PSEUDOMONAS**-SYRINGAE; FROST INJURY; FROZEN **FOODS**; KINU-TOFU

=> dis L11 1-23 abs kwic

L11 ANSWER 1 OF 23 CAPLUS COPYRIGHT 2004 ACS on STN
AB A method of designing an **antifreeze protein** or peptide from a repeat sequence of an ice nucleation protein and then effecting, is disclosed. Use of the **antifreeze protein** as ice crystal formation inhibitor and f.p. lowering agent, and ice slurry containing it, are claimed. An artificial 96-amino acid protein (INP96) was designed based on a repeat sequence from **Pseudomonas** syringae ice nucleation protein, InaZ. Bipyramidal ice crystals were formed in a solution containing INP96.
TI Designing **antifreeze proteins** from a tandem repeat in the **Pseudomonas** syringae ice nucleation protein
AB A method of designing an **antifreeze protein** or peptide from a repeat sequence of an ice nucleation protein and then effecting, is disclosed. Use of the **antifreeze protein** as ice crystal formation inhibitor and f.p. lowering agent, and ice slurry containing it, are claimed. An artificial 96-amino acid protein (INP96) was designed based on a repeat sequence from **Pseudomonas** syringae ice nucleation protein, InaZ. Bipyramidal ice crystals were formed in a solution

containing INP96.

ST **antifreeze protein Pseudomonas** ice nucleation cDNA sequence; **Pseudomonas** ice nucleation protein gene sequence

IT Proteins
RL: BPN (Biosynthetic preparation); BSU (Biological study, unclassified); NUU (Other use, unclassified); BIOL (Biological study); PREP (Preparation); USES (Uses)
(antifreeze; designing **antifreeze proteins** from a tandem repeat in **Pseudomonas** syringae ice nucleation protein)

IT Genetic engineering
Molecular cloning
Protein engineering
Protein sequences
Pseudomonas syringae
Repeat motifs (protein)
(designing **antifreeze proteins** from a tandem repeat in **Pseudomonas** syringae ice nucleation protein)

IT Crystal nucleation
(ice, inhibitor; designing **antifreeze proteins** from a tandem repeat in **Pseudomonas** syringae ice nucleation protein)

IT Proteins
RL: BSU (Biological study, unclassified); NUU (Other use, unclassified); BIOL (Biological study); USES (Uses)
(ice-nucleating, InaZ; designing **antifreeze proteins** from a tandem repeat in **Pseudomonas** syringae ice nucleation protein)

IT Escherichia coli
(recombinant expression in; designing **antifreeze proteins** from a tandem repeat in **Pseudomonas** syringae ice nucleation protein)

IT Antifreeze
(use as; designing **antifreeze proteins** from a tandem repeat in **Pseudomonas** syringae ice nucleation protein)

IT 744733-15-1 744733-16-2 744733-17-3, **Antifreeze protein** INP96 (synthetic)
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(amino acid sequence; designing **antifreeze proteins** from a tandem repeat in **Pseudomonas** syringae ice nucleation protein)

IT 744733-71-9 744733-72-0 744733-73-1 744733-74-2 744733-75-3 744733-76-4 744733-77-5
RL: PRP (Properties)
(unclaimed nucleotide sequence; designing **antifreeze proteins** from a tandem repeat in the **Pseudomonas** syringae ice nucleation protein)

IT 744733-68-4 744733-69-5 744733-70-8 744733-78-6 744733-79-7 744733-80-0
RL: PRP (Properties)
(unclaimed protein sequence; designing **antifreeze proteins** from a tandem repeat in the **Pseudomonas** syringae ice nucleation protein)

L11 ANSWER 2 OF 23 CAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 1

AB The Arctic plant growth-promoting rhizobacterium **Pseudomonas** putida GR12-2 secretes an **antifreeze protein** (AFP) that promotes survival at subzero temps. The AFP is unusual in that it also exhibits a low level of ice nucleation activity. A DNA fragment with an open reading frame encoding 473 amino acids was cloned by PCR and inverse PCR using primers designed from partial amino acid sequences of the isolated AFP. The predicted gene product, AfpA, had a mol. mass of 47.3 kDa, a pI of 3.51, and no previously known function. Although AfpA is a secreted protein, it lacked an N-terminal signal peptide and was

shown by sequence anal. to have two possible secretion systems: a hemolysin-like, calcium-binding secretion domain and a type V autotransporter domain found in gram-neg. bacteria. Expression of afpA in Escherichia coli yielded an intracellular 72-kDa protein modified with both sugars and lipids that exhibited lower levels of antifreeze and ice nucleation activities than the native protein. The 164-kDa AFP previously purified from P. putida GR12-2 was a lipoglycoprotein, and the carbohydrate was required for ice nucleation activity. Therefore, the recombinant protein may not have been properly posttranslationally modified. The AfpA sequence was most similar to cell wall-associated proteins and less similar to ice nucleation proteins (INPs). Hydropathy plots revealed that the amino acid sequence of AfpA was more hydrophobic than those of the INPs in the domain that forms the ice template, thus suggesting that AFPs and INPs interact differently with ice. To our knowledge, this is the first gene encoding a protein with both antifreeze and ice nucleation activities to be isolated and characterized.

TI Cloning and expression of afpA, a gene encoding an **antifreeze protein** from the arctic plant growth-promoting rhizobacterium **Pseudomonas** putida GR12-2

AB The Arctic plant growth-promoting rhizobacterium **Pseudomonas** putida GR12-2 secretes an **antifreeze protein** (AFP) that promotes survival at subzero temps. The AFP is unusual in that it also exhibits a low level of. . .

L11 ANSWER 3 OF 23 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN

AB **Antifreeze proteins** (AFPs) are a structurally diverse group of proteins that have the ability to modify ice crystal structure and inhibit recrystallization of ice. AFPs are well characterized in fish and insects, but very few bacterial species have been shown to have AFP activity to date. Thirty eight freshwater to hypersaline lakes in the Vestfold Hills and Larsemann Hills of Eastern Antarctica were sampled for AFPs during 2000. Eight hundred and sixty six bacterial isolates were cultivated. A novel AFP assay, designed for high-throughput analysis in Antarctica, demonstrated putative activity in 187 of the cultures. Subsequent analysis of the putative positive isolates showed 19 isolates with significant recrystallization inhibition (RI) activity. The 19 RI active isolates were characterized using ARDRA (amplified rDNA restriction analysis) and 16S rDNA sequencing. They belong to genera from the alpha- and gamma-Proteobacteria, with genera from the gamma-subdivision being predominant. The 19 AFP-active isolates were isolated from four physico-chemically diverse lakes. Ace Lake and Oval Lake were both meromictic with correspondingly characteristic chemically stratified water columns. Pendant Lake was a saline holomictic lake with different chemical properties to the two meromictic lakes. Triple Lake was a hypersaline lake rich in dissolved organic carbon and inorganic nutrients. The environments from which the AFP-active isolates were isolated are remarkably diverse. It will be of interest, therefore, to elucidate the evolutionary forces that have led to the acquisition of functional AFP activity in microbes of the Vestfold Hills lakes and to discover the role the antifreezes play in these organisms.

TI Demonstration of **antifreeze protein** activity in Antarctic lake bacteria

AB **Antifreeze proteins** (AFPs) are a structurally diverse group of proteins that have the ability to modify ice crystal structure and inhibit recrystallization. . .

STP KeyWords Plus (R): THERMAL HYSTERESIS PROTEIN; **PSEUDOMONAS** -PUTIDA GR12-2; RICH-REPEAT PROTEIN; ICE RECRYSTALLIZATION; FREEZING RESISTANCE; SALINE LAKES; IDENTIFICATION; PLANT; ACINETOBACTER; INHIBITION

L11 ANSWER 4 OF 23 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN DUPLICATE 2

AB Three substances have been tested for ice nucleation inhibition. These were an **antifreeze protein** AFP III from the fish Macrozoarces americanus, an antifreeze glycoprotein AFGP from the fish

Dissostichus mawsoni, and an 80% hydrolysed poly(vinyl alcohol) with a molecular weight of 9 to 10 kD. A nucleation spectrometer was used to test nucleation inhibition at a range of concentrations against two types of ice nuclei: those present in tap water and a bacterial nucleator from **Pseudomonas** syringae. The PVA reduced the nucleation temperature of tap water and the bacterial dispersions at all the concentrations which were tested. The AFGP reduced the nucleation temperature of tap water but enhanced the nucleation activity of the bacterial nucleators. At low concentrations the AFP III reduced the nucleation temperature of both tap water and the bacterial nucleator. At high concentrations the AFP III enhanced the nucleation temperature of the bacterial nucleator and broadened the nucleation spectrum of the tap water to encompass the nucleation spread of the control. The possible mechanisms of nucleation suppression and enhancement are discussed.

TI The effect of **antifreeze proteins** and poly(vinyl alcohol) on the nucleation of ice: A preliminary study.

AB Three substances have been tested for ice nucleation inhibition. These were an **antifreeze protein** AFP III from the fish Macrozoarces americanus, an antifreeze glycoprotein AFGP from the fish Dissostichus mawsoni, and an 80% hydrolysed. . . . a range of concentrations against two types of ice nuclei: those present in tap water and a bacterial nucleator from **Pseudomonas** syringae. The PVA reduced the nucleation temperature of tap water and the bacterial dispersions at all the concentrations which were. . .

IT Major Concepts
Biochemistry and Molecular Biophysics; Methods and Techniques

IT Chemicals & Biochemicals
AFGP; AFP III; **antifreeze protein**; ice: nucleation; poly(vinyl alcohol)

ORGN . . .
Fish, Nonhuman Vertebrates, Vertebrates

ORGN Classifier
Pseudomonadaceae 06508

Super Taxa
Gram-Negative Aerobic Rods and Cocci; Eubacteria; Bacteria; Microorganisms

Organism Name
Pseudomonas syringae (species)

Taxa Notes
Bacteria, Eubacteria, Microorganisms

L11 ANSWER 5 OF 23 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN

AB Many organisms have evolved into unique mechanisms which minimize freezing injury due to extracellular ice formation. Specifically, certain bacteria have produced a few proteins each with different functions. For example, the ice nucleation protein acts as a template for ice formation, which is responsible for imparting ice nucleating activity. The anti-nucleating protein inhibits the fluctuation of ice nucleus formation by a foreign particle in the water drop. Also, the! **antifreeze proteins** depress the freezing temperature, modify or suppress ice crystal growth, inhibit ice recystallization, and protect the cell membrane from cold-induced damage. In this article, a review on the current knowledge of the structure and the function of these three types of proteins, which are capable of interacting with ice itself or its nuclei from bacteria.

AB . . . The anti-nucleating protein inhibits the fluctuation of ice nucleus formation by a foreign particle in the water drop. Also, the! **antifreeze proteins** depress the freezing temperature, modify or suppress ice crystal growth, inhibit ice recystallization, and protect the cell membrane from cold-induced. . .

ST Author Keywords: **antifreeze protein**; ice-nucleating protein; anti-nucleating protein; Antarctica

STP KeyWords Plus (R): **PSEUDOMONAS**-FLUORESCENS KUIN-1; ERWINIA-UREDOVORA KUIN-3; NUCLEATION ACTIVE GENE; **ANTIFREEZE**

PROTEIN; ANTARCTIC ORIGIN; LOW-TEMPERATURE; PUTIDA GR12-2; IDENTIFICATION; NUCLEI; EXPRESSION

L11 ANSWER 6 OF 23 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN

AB We found six bacteria capable of producing **antifreeze protein** (AFP) from Ross Island, Antarctica. Among these AFP-producing bacteria, strain Number 82 had the highest antifreeze activity and was identified as Moraxella sp. The optimum temperature and pH for the production of AFP were 5degreesC and 7.0, respectively. After partially purifying the AFP from the culture supernatant using 60% saturation of ammonium sulfate, only the 52-kDa protein band (100 mug/ml) which eluted from SDS-PAGE indicated antifreeze activity by the formation of hexagonal crystals. Furthermore, we confirmed that this AFP was a lipoprotein by the lipid stain test and treatment with some enzymes and that it had no ice-nucleating activity. Also, the N-terminal amino acid sequence of this AFP had high similarity with that of outer membrane proteins from Moraxella (Branhamella) catarrhalis. This is the first report of AFP-producing bacteria in Antarctica and an antifreeze lipoprotein (AFLP) from Moraxella sp.

AB We found six bacteria capable of producing **antifreeze protein** (AFP) from Ross Island, Antarctica. Among these AFP-producing bacteria, strain Number 82 had the highest antifreeze activity and was identified. . .

ST Author Keywords: **antifreeze protein**; Antarctica; Moraxella

STP KeyWords Plus (R): **PSEUDOMONAS**-PUTIDA GR12-2; BRANHAMELLA-CATARRHALIS; LOW-TEMPERATURE; COLD SHOCK; PROTEIN; NUCLEATION; ADAPTATION; BACTERIUM; PRECURSOR; SURVIVAL

L11 ANSWER 7 OF 23 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN

AB This paper reviews new developments in methods of freezing (high-pressure freezing, dehydrofreezing and applications of **antifreeze protein** and ice nucleation protein) and thawing (high-pressure and microwave thawing, ohmic thawing and acoustic thawing) for foods. With a good understanding of the solid-liquid phase diagram of water, the effects of pressure on food freezing-thawing cycles are highlighted. High-pressure freezing promotes uniform and rapid ice nucleation and growth through the whole sample. Dehydrofreezing has been successfully used in freezing of vegetables and fruits with the advantage of less damage to plant texture because of partial water removal before freezing. Recently, studies have been carried out for the biotechnological use of antifreeze and ice nucleation proteins because of their uniqueness in directly improving freezing processes. Thawing under pressure can be achieved at lower temperature than that at atmospheric pressures. Finally microwave, ohmic and acoustic thawing are described. It is hoped that this paper will attract more research in novel freezing and thawing processes and methods. (C) 2002 Elsevier Science Ltd. All rights reserved.

AB This paper reviews new developments in methods of freezing (high-pressure freezing, dehydrofreezing and applications of **antifreeze protein** and ice nucleation protein) and thawing (high-pressure and microwave thawing, ohmic thawing and acoustic thawing) for foods. With a good. . .

ST Author Keywords: acoustic; **antifreeze protein**; dehydrofreezing; freezing; freezing time; foods; high pressure; ice crystal; ice nucleation protein; microwave; ohmic; thawing

STP KeyWords Plus (R): HIGH HYDROSTATIC-PRESSURE; BACTERIAL ICE NUCLEATION; **ANTIFREEZE PROTEINS**; ERWINIA-HERBICOLA; TEXTURAL QUALITY; OSMOTIC CONCENTRATION; **PSEUDOMONAS**-SYRINGAE; FROST INJURY; FROZEN FOODS; KINU-TOFU

L11 ANSWER 8 OF 23 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN DUPLICATE 3

AB **Antifreeze proteins** depress the freezing point of

water while not affecting the melting point, producing a characteristic difference in freezing and melting points termed thermal hysteresis. Larvae of the beetle Dendroides canadensis accumulate potent **antifreeze proteins** (DAFPs) in their hemolymph and gut, but to achieve high levels of thermal hysteresis requires enhancers, such as glycerol. DAFPs have previously been shown to inhibit the activity of bacterial and hemolymph protein ice nucleators, however, the effect was not large and therefore the effectiveness of the DAFPs in promoting supercooling of the larvae in winter was doubtful. However, this study demonstrates that DAFPs, in combination with the thermal hysteresis enhancers glycerol (1 M) or citrate (0.5 M), eliminated the activity of hemolymph protein ice nucleators and **Pseudomonas** syringae ice-nucleating active bacteria, and lowered the supercooling points (nucleation temperatures) of aqueous solutions containing these ice nucleators to those of water or buffer alone. This shows that the DAFPs, along with glycerol, play a critical role in promoting hemolymph supercooling in overwintering D. canadensis. Also, DAFPs in combination with enhancers may be useful in applications which require inhibition of ice nucleators.

TI The inhibition of ice nucleators by insect **antifreeze proteins** is enhanced by glycerol and citrate.

AB **Antifreeze proteins** depress the freezing point of water while not affecting the melting point, producing a characteristic difference in freezing and melting points termed thermal hysteresis. Larvae of the beetle Dendroides canadensis accumulate potent **antifreeze proteins** (DAFPs) in their hemolymph and gut, but to achieve high levels of thermal hysteresis requires enhancers, such as glycerol. DAFPs. . . the thermal hysteresis enhancers glycerol (1 M) or citrate (0.5 M), eliminated the activity of hemolymph protein ice nucleators and **Pseudomonas** syringae ice-nucleating active bacteria, and lowered the supercooling points (nucleation temperatures) of aqueous solutions containing these ice nucleators to those. . .

IT . . .
Biochemistry and Molecular Biophysics; Metabolism

IT Parts, Structures, & Systems of Organisms
hemolymph: blood and lymphatics

IT Chemicals & Biochemicals
antifreeze proteins; citrate; glycerol; protein ice nucleators

L11 ANSWER 9 OF 23 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN DUPLICATE 4

AB A novel cDNA clone, Tad1, was isolated from crown tissue of winter wheat after differential screening of cold acclimation-induced genes. The Tad1 cDNA encoded a 23 kDa polypeptide with a potential N-terminal signal sequence. The putative mature sequence showed striking similarity to plant defensins or γ-thionins, representing low molecular size antipathogenic polypeptides. High levels of Tad1 mRNA accumulation occurred within one day of cold acclimation in crown tissue and the level was maintained throughout 14 days of cold acclimation. Similar rapid induction was observed in young seedlings treated with low temperature but not with exogenous abscisic acid. In contrast to defensins from other plant species, neither salicylic acid nor methyl jasmonate induced expression of Tad1. The recombinant mature form of TAD1 polypeptide inhibited the growth of the phytopathogenic bacteria, **Pseudomonas** cichorii; however, no antifreeze activity was detected. Collectively, these data suggested that Tad1 is induced in cold-acclimated winter wheat independent of major defense signaling(s) and is involved in low temperature-induced resistance to pathogens during winter hardening.

AB . . . methyl jasmonate induced expression of Tad1. The recombinant mature form of TAD1 polypeptide inhibited the growth of the phytopathogenic bacteria, **Pseudomonas** cichorii; however, no antifreeze activity was detected. Collectively, these data suggested that Tad1 is induced in cold-acclimated winter wheat independent. . .

CT Medical Descriptors:
*cold acclimatization
*Tad1 gene
*gene
plant genetics
winter wheat
gene induction
molecular cloning
gene isolation
plant tissue
genetic screening
amino terminal sequence
genetic code
sequence homology
molecular weight
protein function
plant seed
low temperature
gene expression
bacterial growth
growth inhibition
Pseudomonas
signal transduction
cold tolerance
antibacterial activity
nonhuman
controlled study
article
nucleotide sequence
priority journal
*defensin
complementary DNA
thionine
messenger RNA: EC, endogenous compound
abscisic acid
salicylic acid
jasmonic acid
recombinant protein
antifreeze protein
protein TAD1
polypeptide
unclassified drug

L11 ANSWER 10 OF 23 CAPLUS COPYRIGHT 2004 ACS on STN
AB The invention relates to a process for preparing a novel anti-freeze peptide and to the peptides obtained from bacteria from an aqueous low-temperature environment, such as Marinomonas protea and a novel **Pseudomonas species.** These anti-freeze peptides can suitably be incorporated in frozen food products such as frozen vegetables and frozen confectionery such as ice cream.
TI Processes and organisms for the production of **anti-freeze proteins**
AB . . . peptide and to the peptides obtained from bacteria from an aqueous low-temperature environment, such as Marinomonas protea and a novel **Pseudomonas species.** These anti-freeze peptides can suitably be incorporated in frozen food products such as frozen vegetables and frozen confectionery such as. . .
ST **antifreeze protein** fermn Marinomonas **Pseudomonas**; food **antifreeze protein**; sequence **antifreeze protein**
IT Ice cream
(**anti-freeze proteins** for use in)
IT Proteins, specific or class
RL: BMF (Bioindustrial manufacture); BPN (Biosynthetic preparation); FFD (Food or feed use); PRP (Properties); PUR (Purification or recovery); BIOL

(Biological study); PREP (Preparation); USES (Uses)
(antifreeze; processes and organisms for the production of **anti-freeze proteins**)

IT Vegetable
(frozen; **anti-freeze proteins** for use in)

IT Fermentation
Marinomonas protea
Pseudomonas synxantha
(processes and organisms for the production of **anti-freeze proteins**)

IT Frozen foods
(vegetables; **anti-freeze proteins** for use in)

IT 346420-90-4P
RL: BMF (Bioindustrial manufacture); BPN (Biosynthetic preparation); FFD (Food or feed use); PRP (Properties); PUR (Purification or recovery); BIOL (Biological study); PREP (Preparation); USES (Uses)
(amino acid sequence; processes and organisms for the production of **anti-freeze proteins**)

IT 344824-54-0 346011-36-7
RL: PRP (Properties)
(nucleotide sequence; processes and organisms for the production of **anti-freeze proteins**)

IT 344824-56-2 346013-83-0, 9: PN: WO0144275 FIGURE: 3 unclaimed DNA
RL: PRP (Properties)
(unclaimed nucleotide sequence; processes and organisms for the production of **anti-freeze proteins**)

IT 225675-43-4 344824-55-1
RL: PRP (Properties)
(unclaimed sequence; processes and organisms for the production of **anti-freeze proteins**)

L11 ANSWER 11 OF 23 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN DUPLICATE 5

AB **Antifreeze proteins** (AFPs) inhibit the growth of ice, whereas ice-nucleation proteins (INPs) promote its formation. Although the structures of several AFPs are known, the structure of INP has been modeled thus far because of the difficulty in determining membrane protein structures. Here, we present a novel model of an INP structure from **Pseudomonas** syringae based on comparison with two newly determined insect AFP structures. The results suggest that both this class of AFPs and INPs may have a similar beta-helical fold and that they could interact with water through the repetitive TXT motif. By theoretical arguments, we show that the distinguishing feature between an ice inhibitor and an ice nucleator lies in the size of the ice-interacting surface. For INPs, the larger surface area acts as a template that is larger than the critical ice embryo surface area required for growth. In contrast, AFPs are small enough so that they bind to ice and inhibit further growth without acting as a nucleator.

TI Modeling **Pseudomonas** syringae ice-nucleation protein as a beta-helical protein.

AB **Antifreeze proteins** (AFPs) inhibit the growth of ice, whereas ice-nucleation proteins (INPs) promote its formation. Although the structures of several AFPs are. . . because of the difficulty in determining membrane protein structures. Here, we present a novel model of an INP structure from **Pseudomonas** syringae based on comparison with two newly determined insect AFP structures. The results suggest that both this class of AFPs. . .

IT Major Concepts
Biochemistry and Molecular Biophysics; Microbiology

IT Chemicals & Biochemicals
antifreeze protein; ice: growth; ice-nucleation protein: beta-helical protein

ORGN Classifier
Pseudomonadaceae 06508

Super Taxa
Gram-Negative Aerobic Rods and Cocci; Eubacteria; Bacteria; Microorganisms
Organism Name
Pseudomonas syringae
Taxa Notes
Bacteria, Eubacteria, Microorganisms

L11 ANSWER 12 OF 23 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN

AB The influence of plant growth promoting (PGP) activity of bacterial communities recovered from each of six weed species (barnyard grass (Echinochloa crusfalli (L.) Beauv.), corn spurrey (Spergula arvensis L.), goldenrod (Sonchus sp.), Italian ryegrass (Lolium multiflorum L.), lamb's-quarters (Chenopodium album L.), and quack grass (Agropyron repens (L.) Beauv.)) was examined in relation to the effect it had on the growth of the potato cultivar Russet Burbank. Bacterial species composition and community structure were compared, species-abundance relationships were determined, and those members conferring positive benefits for potato growth and development were identified. Of the genera identified, Bacillus, Arthrobacter, Stenotrophomonas, Acinetobacter, and **Pseudomonas** were the most common, and Stenotrophomonas maltophilia was the most frequent species recovered across all sources. Significantly higher population densities were found in the root zones of quack grass, compared with Italian ryegrass and lamb's-quarters. There were no significant differences in species richness among the root zones; however, evenness indices (species distribution) were significantly lower in corn spurrey ($P = 0.05$). Significantly higher diversity indices (Hill-1 and Hill-2 numbers) ($P = 0.05$) were found in the root zone soil communities of potato and goldenrod, indicating a decrease in the proportional abundance of common and very abundant species, respectively, while in barnyard grass, corn spurrey, and Italian ryegrass the reverse was the case. In both years of the study, Italian ryegrass and corn spurrey were consistently better sources of PGP rhizobacteria for potatoes, significantly ($P < 0.001$) increasing the mean wet weight of shoots and roots in in vitro bacterization studies. Barnyard grass was a consistently poor source of such isolates. Species-abundance measures of root zone bacterial biodiversity were not found, in this instance, to be a particularly good predictor of the presence or absence of PGP rhizobacteria. We consider that the study of complementary crops and soil-conditioning treatments should not preclude the examination of weed species as possible beneficials, as alterations in rhizobacterial biodiversity and functional versatility can influence the numbers and types of PGP bacterial strains, and consequently may serve to improve soil quality.

AB . . . members conferring positive benefits for potato growth and development were identified. Of the genera identified, Bacillus, Arthrobacter, Stenotrophomonas, Acinetobacter, and **Pseudomonas** were the most common, and Stenotrophomonas maltophilia was the most frequent species recovered across all sources. Significantly higher population densities. . .

STP KeyWords Plus (R): **PSEUDOMONAS**-PUTIDA GR12-2; RED-CLOVER; SYSTEMIC RESISTANCE; RHIZOSPHERE BACTERIA; ENDOPHYTIC BACTERIA; **ANTIFREEZE PROTEIN**; MICROORGANISMS; BIODIVERSITY; INOCULATION; STIMULATION

L11 ANSWER 13 OF 23 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN DUPLICATE 6

AB Following transposon Tn5 mutagenesis of the plant growth-promoting rhizobacterium **Pseudomonas** putida GR12-2, mutants that have different freeze-resistant properties were selected. Five of the freeze-sensitive mutants, i.e. FSM-5, -6, -14, -29, and -41, secreted a lower amount of **antifreeze protein**-(AFP) into the culture broth compared with the wild-type. Among of these five mutants, the three mutants (FSM-6, FSM-14, and FSM-41) that have the lowest level

of freezing resistance (4.0-6.0% survival) also produce AFP at low levels (0.5-0.9 mug/mL) compared with the wild-type (4.8 mug/ml). The antifreeze and ice-nucleating activities of the AFP from these three mutant strains were similar to those of wild-type. Furthermore, the decreased freezing resistance from three mutants could be partially restored by adding purified AFP to mutant cell suspensions. Freezing resistance of three mutants was found to increase in proportion to the addition of AFP up to a concentration of 50 mug/mL. We conclude that accumulation of AFP is one component of the mechanism for freezing resistance in bacteria.

TI Relationship between **antifreeze protein** and freezing resistance in **Pseudomonas** putida GR12-2.

AB Following transposon Tn5 mutagenesis of the plant growth-promoting rhizobacterium **Pseudomonas** putida GR12-2, mutants that have different freeze-resistant properties were selected. Five of the freeze-sensitive mutants, i.e. FSM-5, -6, -14, -29, and -41, secreted a lower amount of **antifreeze protein**-(AFP) into the culture broth compared with the wild-type. Among of these five mutants, the three mutants (FSM-6, FSM-14, and FSM-41). . .

IT Major Concepts
Cell Biology; Molecular Genetics (Biochemistry and Molecular Biophysics)

IT Chemicals & Biochemicals
antifreeze protein [AFP]: antifreeze activity, ice-nucleating activity

ORGN Classifier
Pseudomonadaceae 06508

Super Taxa
Gram-Negative Aerobic Rods and Cocci; Eubacteria; Bacteria; Microorganisms

Organism Name
Pseudomonas putida: strain-FSM-14, strain-FSM-29, strain-FSM-41, strain-FSM-5, strain-FSM-6, strain-GR12-2

Taxa Notes
Bacteria, Eubacteria, Microorganisms

L11 ANSWER 14 OF 23 CAPLUS COPYRIGHT 2004 ACS on STN

AB A review with 6 refs. on ice nucleation proteins isolated from bacteria (**Pseudomonas**, Erwinia, etc.) and **antifreeze proteins** isolated from plants and bacteria, and biol. roles of the proteins for adaptation to cold conditions.

AB A review with 6 refs. on ice nucleation proteins isolated from bacteria (**Pseudomonas**, Erwinia, etc.) and **antifreeze proteins** isolated from plants and bacteria, and biol. roles of the proteins for adaptation to cold conditions.

ST cold adaptation plant bacteria ice review; ice nucleation **antifreeze protein** cold adaptation plant bacteria review

L11 ANSWER 15 OF 23 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN

AB Freeze injury is one of the main limiting factors to crop production and distribution of horticultural crops. Despite the numerous research efforts devoted to reduce freezing injury, it still accounts for greater losses of fruits and vegetables than any other environmental or biological hazard and the ultimate causes determining cold hardiness remain uncertain. In temperate climates, important damages on deciduous fruit trees are produced in buds, flowers and developing fruits after dormancy and losses due to frosts during bloom are usually more important than those due to low winter temperatures. The information available on the influence of late spring frosts on reproductive organs of deciduous fruit trees in temperate climates is reviewed. The freezing process is examined, paying attention to how subsequent frost damage is caused at the cellular level as well as its anatomical and morphological consequences in flowers and fruits. The flower hardiness response is evaluated in terms of genotype, phenology and a number of physiological aspects as the formation of ice nucleation, moisture content and nutritive status. Finally, new

perspectives are explored. (C) 2000 Elsevier Science B.V. All rights reserved.

STP KeyWords Plus (R): ACTIVE **PSEUDOMONAS**-SYRINGAE; ICE-NUCLEATION; FREEZING TOLERANCE; LOW-TEMPERATURE; COLD-ACCLIMATION; **ANTIFREEZE PROTEINS**; SEASONAL-VARIATION; XYLEM DEVELOPMENT; WOODY-PLANTS; WINTER RYE

L11 ANSWER 16 OF 23 CAPLUS COPYRIGHT 2004 ACS on STN

AB A review with 8 refs. Biol. macromols. which regulate f.p. of water have high potential for industrial applications. Proteins such as ice-nucleation protein (INP) and **antifreeze protein** (AFP) are members of such mols., which could be obtained from nature. In order to understand the mechanism of their f.p. regulations, elucidation of three-dimensional structure of INP nd AFP is crucial. We have examined three-dimensional structure of proteins by using NMR (NMR) spectroscopy and x-ray crystallog. In this review, we describe about three-dimensional structure of INP and AFP on the basis of the NMR exptl. results. A central domain of iNP derived from **Pseudomonas** sp. is thought to be a nucleation site for the freezing, comprising tandem repeats of high-fidelity consensus sequence. We have determined the three-dimensional structure of the peptide fragments having various length and sequence, and found that hexapeptide segment forms a typical hairpin-loop conformation. It is suggested from this result that the unit structure of the tandem repeat region is made up with 16 residues, and the model of the three-dimensional structure of the central domain can be constructed from the repetition of this unit structure. RD3, AFP of Lycodichthys dearborni, is composed of the 2 domains, both of which inhibit growth of ice-crystal, having high homol. in amino acid sequence with each other. The three-dimensional structure of the N-terminal half domain of RD3 (RD3-N1) with linker potion connecting the C-terminal domain was determined It was found that ice-binding site of RD3-N1 located in one side of the mol. so as to form a flat surface, which will allow complementary interaction between RD3-N1 and water mols. of ice. Furthermore, structure of linker portion was determined and found to be rigid. On the basis of these results, whole architecture of intact RD3 mol. could be proposed.

AB . . . Biol. macromols. which regulate f.p. of water have high potential for industrial applications. Proteins such as ice-nucleation protein (INP) and **antifreeze protein** (AFP) are members of such mols., which could be obtained from nature. In order to understand the mechanism of their. . . structure of INP and AFP on the basis of the NMR exptl. results. A central domain of iNP derived from **Pseudomonas** sp. is thought to be a nucleation site for the freezing, comprising tandem repeats of high-fidelity consensus sequence. We have. . .

L11 ANSWER 17 OF 23 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN DUPLICATE 7

AB An **antifreeze protein** secreted to the growth medium by the plant growth promoting rhizobacterium **Pseudomonas** putida GR12-2 was purified to apparent homogeneity. The purified protein has a molecular mass of 164+-15 kDa and an isoelectric point of 5.3, contains both carbohydrate and lipid moieties, and is relatively rich in glycine and alanine. The properties of the purified **antifreeze protein** are similar to the properties previously reported for bacterial ice-nucleation proteins. In fact, the purified **antifreeze protein** also displays a low level of ice-nucleation activity. Removal of approximately 92 kDa of carbohydrate from the 164-kDa antifreeze glycoprotein did not noticeably alter the antifreeze activity of the molecule, although it did diminish the ice-nucleation activity. This is the first report of an **antifreeze protein** that also is active as an ice-nucleation protein.

TI Isolation and characterization of an **antifreeze protein** with ice nucleation activity from the plant growth promoting rhizobacterium **Pseudomonas** putida GR12-2.

AB An **antifreeze protein** secreted to the growth medium by the plant growth promoting rhizobacterium **Pseudomonas** putida GR12-2 was purified to apparent homogeneity. The purified protein has a molecular mass of 164+-15 kDa and an isoelectric. . . 5.3, contains both carbohydrate and lipid moieties, and is relatively rich in glycine and alanine. The properties of the purified **antifreeze protein** are similar to the properties previously reported for bacterial ice-nucleation proteins. In fact, the purified **antifreeze protein** also displays a low level of ice-nucleation activity. Removal of approximately 92 kDa of carbohydrate from the 164-kDa antifreeze glycoprotein. . . the antifreeze activity of the molecule, although it did diminish the ice-nucleation activity. This is the first report of an **antifreeze protein** that also is active as an ice-nucleation protein.

IT Major Concepts
Biochemistry and Molecular Biophysics

IT Chemicals & Biochemicals
antifreeze protein: ice nucleation activity

ORGN . . .
plant
Taxa Notes
Plants

ORGN Classifier
Pseudomonadaceae 06508
Super Taxa
Gram-Negative Aerobic Rods and Cocci; Eubacteria; Bacteria; Microorganisms
Organism Name
Pseudomonas-putida: strain-GR12-2
Taxa Notes
Bacteria, Eubacteria, Microorganisms

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AB Psychrophilic organisms have successfully colonized polar and alpine regions and are able to grow efficiently at sub-zero temperatures. At the enzymatic level, such organisms have to cope with the reduction of chemical reaction rates induced by low temperatures in order to maintain adequate metabolic fluxes. Thermal compensation in cold-adapted enzymes is reached through improved turnover number and catalytic efficiency. This optimization of the catalytic parameters can originate from a highly flexible structure which provides enhanced abilities to undergo conformational changes during catalysis. Thermal instability of cold-adapted enzymes is therefore regarded as a consequence of their conformational flexibility. A survey of the psychrophilic enzymes studied so far reveals only minor alterations of the primary structure when compared to mesophilic or thermophilic homologues. However, all known structural factors and weak interactions involved in protein stability are either reduced in number or modified in order to increase their flexibility.

STP KeyWords Plus (R): ALTEROMONAS-HALOPLANCTIS A23; ALPHA-AMYLASE; ATLANTIC COD; PSYCHROTROPHIC BACTERIUM; **PSEUDOMONAS**-FLUORESCENS; EVOLUTIONARY ADAPTATION; ANGSTROM RESOLUTION; NUCLEOTIDE-SEQUENCE; **ANTIFREEZE PROTEIN**; ANTARCTIC BACTERIA

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AB The effect of gut fluid ice nucleators and **antifreeze proteins** on maintenance of supercooling was explored in fire-colored beetle larvae, Dendroides canadensis, via seasonal monitoring of supercooling points, **antifreeze protein** activity and ice nucleator activity of gut fluid and/or larvae. During cold hardening in the field, freeze-avoiding larvae evacuated their guts and depressed larval supercooling points. Analysis of gut fluid indicated supercooling points and ice nucleator activity decreased, whereas

antifreeze protein activity increased as winter approached. Suspensions of bacteria isolated from guts of feeding larvae collected in spring/summer had higher supercooling points than those from midwinter-collected non-feeding larvae, suggesting bacterial ice nucleators are removed from midwinter gut fluid. The ice nucleation active bacterium **Pseudomonas** fluorescens was isolated from gut fluid of feeding larvae but was absent in winter. When mixed with purified D. canadensis hemolymph **antifreeze proteins** (structurally similar and/or identical to those in gut fluid), the cumulative ice nucleus spectra of P. fluorescens suspensions were shifted to lower temperatures indicating an inhibitory effect on the bacteria's ice-nucleating phenotype. By extending larval supercooling capacity, both gut clearing and masking of bacterial ice nucleators by **antifreeze proteins** may contribute to overwintering survival in supercooled insects.

TI . . . of the supercooled state in the gut fluid of overwintering pyrochroid beetle larvae, Dendroides canadensis: Role of ice nucleators and **antifreeze proteins.**

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IT Miscellaneous Descriptors
ANTIFREEZE PROTEINS; DIGESTIVE SYSTEM; FREEZE-AVOIDING; GUT CLEARING; GUT FLUID; ICE NUCLEATOR PROTEINS; LARVA; LARVAL SUPERCOOLING POINTS; OVERWINTERING; SUPERCOOLED STATE MAINTENANCE

ORGN . . .
Animals, Arthropods, Insects, Invertebrates

ORGN Classifier
Pseudomonadaceae 06508

Super Taxa
Gram-Negative Aerobic Rods and Cocci; Eubacteria; Bacteria; Microorganisms

Organism Name
Pseudomonas fluorescens

Taxa Notes
Bacteria, Eubacteria, Microorganisms

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AB The plant growth promoting rhizobacterium **Pseudomonas** putida GR12-2 was originally isolated from the rhizosphere of plants growing in the Canadian High Arctic. Here we report that this bacterium was able to grow and promote root elongation of both spring and winter canola at 5 degree C, a temperature at which only a relatively small number of bacteria are able to proliferate and function. In addition, the bacterium

survived exposure to freezing temperatures, i.e., -20 and -50 degree C. In an effort to determine the mechanistic basis for this behaviour, it was discovered that following growth at 5 degree C, P. putida GR12-2 synthesized and secreted to the growth medium a protein with antifreeze activity. Analysis of the spent growth medium, following concentration by ultrafiltration, by SDS-polyacrylamide gel electrophoresis revealed the presence of one major protein with a molecular mass of approximately 32-34 kDa and a number of minor proteins. However, at this point it is not known which of these proteins contains the antifreeze activity.

TI Low temperature growth, freezing survival, and production of **antifreeze protein** by the plant growth promoting rhizobacterium **Pseudomonas** putida GR12-2.

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ORGN . . .

Notes

Bacteria, Eubacteria, Microorganisms

ORGN Classifier

Pseudomonadaceae 06508

Super Taxa

Gram-Negative Aerobic Rods and Cocci; Eubacteria; Bacteria; Microorganisms

Organism Name

Pseudomonas putida

Taxa Notes

Bacteria, Eubacteria, Microorganisms

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TI Does the plant growth-promoting rhizobacterium **Pseudomonas** putida GR12-2 survive cold temperatures by synthesizing its own **antifreeze protein**?.

ORGN Classifier

Pseudomonadaceae 06508

Super Taxa

Gram-Negative Aerobic Rods and Cocci; Eubacteria; Bacteria; Microorganisms

Organism Name

Pseudomonas putida

Taxa Notes

Bacteria, Eubacteria, Microorganisms

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AB Since most insects are unable to survive internal ice formation a key factor in their overwintering survival is the regulation of the temperature at which they spontaneously freeze. To enhance their supercooling capacity overwintering insects eliminate endogenous ice nucleators, accumulate low-molecular-weight polyols and sugars, and synthesize hemolymph **antifreeze proteins.** A number of freeze-tolerant species contain proteins/lipoproteins or insoluble crystals that are ice nucleating active at relatively high subzero temperatures. Only recently have ice nucleating active bacteria and fungi been identified as normal flora in the gut of insects. These microorganisms are the most efficient class of heterogeneous ice nucleators that have been found in insects. Ice nucleating active microorganisms can regulate the supercooling capacity of insects when ingested or applied topically. These unique microorganisms may offer a novel means for the biological control of insect pests during the winter.

AB . . . freeze. To enhance their supercooling capacity overwintering insects eliminate endogenous ice nucleators, accumulate low-molecular-weight polyols and sugars, and synthesize hemolymph **antifreeze proteins.** A number of freeze-tolerant species contain proteins/lipoproteins or insoluble crystals that are ice

nucleating active at relatively high subzero temperatures.. . .
STP KeyWords Plus (R): BEETLE HIPPODAMIA-CONVERGENS; XYLOSTELLA L PUPAE; **PSEUDOMONAS**-SYRINGAE; ERWINIA-HERBICOLA; PLUTELLA-XYLOSTELLA; SUPERCOOLING POINT; DIAMONDBACK MOTH; FROST INJURY; SARCOPHAGA-CRASSIPALPIS; TERRESTRIAL ARTHROPODS

L11 ANSWER 23 OF 23 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN
AB Many organisms have evolved novel mechanisms to minimize freezing injury due to extracellular ice formation. This article reviews our present knowledge on the structure and mode of action of two types of proteins capable of ice interaction. The **antifreeze proteins** inhibit ice crystal formation and alter ice growth habits. The ice nucleation proteins, on the other hand, provide a proper template to stimulate ice growth. The potential applications of these proteins in different industries are discussed.
AB . . . our present knowledge on the structure and mode of action of two types of proteins capable of ice interaction. The **antifreeze proteins** inhibit ice crystal formation and alter ice growth habits. The ice nucleation proteins, on the other hand, provide a proper. . .
STP KeyWords Plus (R): ANTIFREEZE GLYCOPROTEIN; **PSEUDOMONAS** -SYRINGAE; FREEZING RESISTANCE; NUCLEATION ACTIVITY; PEPTIDE ANTIFREEZE; ERWINIA-HERBICOLA; FISH BLOOD; NUCLEI; ADSORPTION; GENE

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L2	2756	(ANTI-FREEZE OR ANTIFREEZE OR ANTI FREEZE) (W) PROTEIN
L3	1	L1 AND L2
L4	307104	PSEUDOMONAS OR PSEUDOMONAS (W) SPECIES
L5	49	L2 AND L4
L6	1403514	FOOD (W) PRODUCT OR FOOD
L7	1403514	(FOOD (W) PRODUCT) OR FOOD
L8	149	L2 AND L7
L9	1	L1 AND L8
L10	2	L4 AND L8
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L13	2	L4 (P) L8

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